Proc. Indian Acad. Sci. (Plant Sci.), Vol. 90, Number 5, October 1981, pp. 395-400.

Modification of sex-expression and fruit-formation on male plants of *Morus nigra* L. by chlorflurenol

V S JAISWAL and ARAVIND KUMAR
Department of Botany, Banaras Hindu University, Varanasi 221 005, India

MS received 24 November 1980; revised 8 May 1981

Abstract. Chlorflurenol (2-Chloro-9-hydroxyfluorene-9-carboxylic acid) applied at 50, 100 and 200 ppm to male plants of *Morus nigra* produced intersex and female flowers. Various transitional stages of male floral primordia into intersex and female flowers were observed. However, the fruits formed by inflorescences with such flowers were abnormal in shape, size and appearance. Frequent drying of inflorescences and fruits occurred at higher concentrations and, as a result, only a few fruits ripened.

Keywords. Chlorflurenol; *Morus nigra* L.; sex-expression.

1. Introduction

Various morphogenetic effects of the morphactin group of chemicals have been reported by several workers (Schneider 1970; Bopp 1972). Recently, interesting effects of these chemicals have been observed on the sex-expression of some monoecious species, e.g., *Cucumis sativus* (Robinson *et al* 1971), *Luffa acutangula* (Bose and Nitsch 1970; Krishnamoorthy 1971; Bisaria 1977), and dioecious species like *Cannabis sativa* (Mohan Ram and Jaiswal 1971). These reports prompted us to evaluate the role of chlorflurenol, a member of the morphactin group of chemicals, on the sex-expression of male plants of a dioecious tree species, *Morus nigra* (black mulberry).

2. Materials and methods

Male plants of *Morus nigra* were selected for treatment. The trees shed all their leaves in the last week of December leaving a number of small buds at each node. The buds subsequently grew and gave rise to new branches bearing inflorescences. The first application of chlorflurenol was made in the first week of January by spraying the plants to run-off level, followed by a second application after two weeks. Three concentrations of the chemical, 50, 100 and 200 ppm, supplemented with 0·1% Triton X-114 as a wetting agent, were used on the experimental plants,

while control plants received only 0·1% Triton X-114. Ten plants were selected for each treatment. The number of inflorescences, bearing flowers of different sex-types, and the fruits formed, were observed and recorded periodically.

3. Results

The chemical retarded the growth of emerging branches and caused severe burning, this effect was directly related to concentration. The branches developing after treatment were stunted with rolled, crumpled and twisted leaves (figure 1 A). Internodal distances became irregular and occasionally, two or more leaves appeared at a single node, particularly at the lowermost node. Some swelling was observed on the new branches.

The newly produced inflorescences on the treated plants with each concentration were severely affected. The size of the newly formed inflorescence was greatly reduced, and it was severely burnt. The lowermost inflorescence was sometimes attached directly at the first node and occasionally present in clusters of more than one (figure 1 A).

The number of inflorescences bearing female flowers was directly related to the concentration of the chemical used (table 1): the number of mixed types of inflorescences with intersex flowers along with the female ones showed an increase at a concentration up to 100 ppm. The higher concentration (200 ppm) was not promotive. These observations suggest that the floral primordia, initially male, change to female by developing a pistil and gradual reduction of anthers. Increase in concentration (from 50 to 200 ppm) of the chemical proportionately increased the rate of change. Hence, at higher concentrations, more male floral primordia changed into female without entering the intersex stage while, at lower concentrations, more primordia remained at the latter stage.

In the intersex flowers, various stages of reduction of stamens, and simultaneous development of pistils were observed (figure 2 A, B). The anthers showed

Table 1. Percentage distribution of inflorescences of different sex-types on the male plants treated with 2-chloro-9-hydroxy fluorene (9) -carboxylic acid (average of 10 plants per treatment).

Concentration (ppm)	Inflorescence with only male flowers (%)	Inflorescence with only female flowers (%)	Inflorescence with intersex, male and/or female flowers (%)
Control	100	...	...
50	77	8	15
100	54	19	27
200	31	46	23

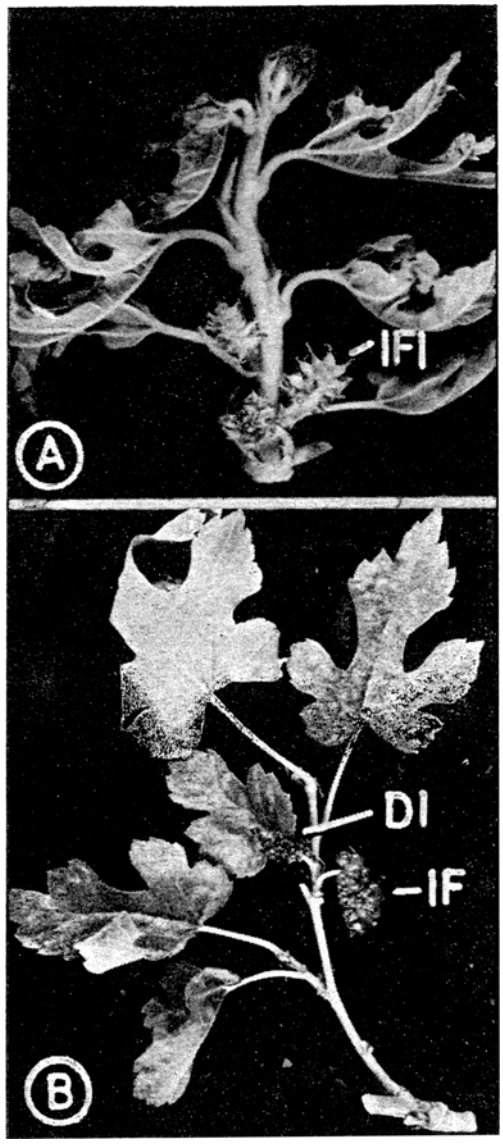

Figure 1. (A) Induced female inflorescence (IFI) on male plant × 1·0. (B) Induced fruit (IF), and a dried inflorescence (DI) × 1·0.

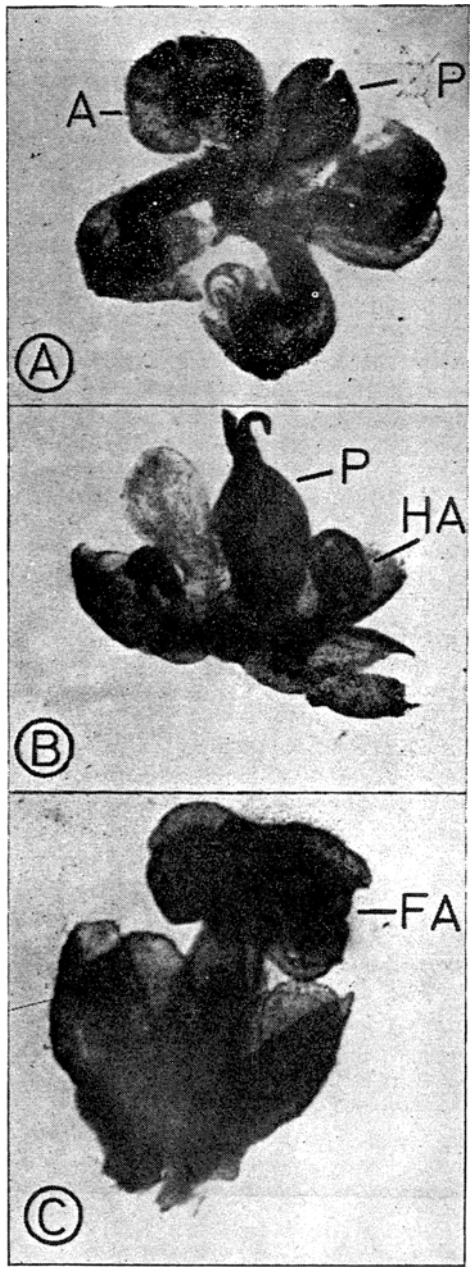

Figure 2. (A) Pistil (P) with affected anthers (A) × 12·2. (B) Pistil (P) with highly-reduced hyaline anthers (HA) × 16·5. (C) An abnormal flower with fused anthers (FA) × 19·6.

various degrees of abnormality with only sterile pollen grains at all stages of meiotic reduction, including those collected from morphologically unaffected flowers. The pollen grains were of various shapes and sizes. *In vitro* germination was not observed when the pollens were cultured in Brewbaker and Kwacks medium (1963). In very advanced stages of reduction the anthers appeared as hyaline membranous structures without any pollen grains (figure 2 B). Several completely-changed female flowers without any trace of male structures were also observed. In some flowers, all the four anthers united (though rarely) with the stigma of the induced pistil making a crown-like structure, and the filaments fused with the wall of the ovary (figure 2 C).

Fruit-setting took place in inflorescences with female and/or intersex flowers (figure 1 B.) Drying of fruits was observed at higher concentration and, as a result, out of 69% fruit-producing inflorescences (female and intersex), at 200 ppm of chlorflurenol, only 8–10% of fruits reached the ripening stage. At 100 ppm, only 46% of the inflorescences produced fruits but only 9–12% ripened. At 50 ppm 4–6% fruits were produced, but there were fewer fruit-forming inflorescences (23%). The fruits formed on the treated plants differed in shape and were, occasionally, sessile. Therefore, while the chemical has a high potentiality for the formation of female flowers, its burning effect reduces the number of fruits capable of ripening.

4. Discussion

In flowering plants, sex-expression is controlled by the optimal balance between endogenous auxin and gibberellin (Atsmon *et al* 1968; Frankel and Galun 1977) and/or between gibberellin and ethylene (Mohan Ram and Jaiswal 1974). Auxin (Heslop-Harrison 1957) and ethylene (Mohan Ram and Jaiswal 1970; Jaiswal and Kumar 1980a) promote femaleness, and gibberellin (Mohan Ram and Jaiswal 1972; Jaiswal and Kumar 1980b) favours maleness in dioecious plants. Increase in auxin/ethylene level and/or decrease in the level of gibberellins induces the development of female flowers on male plants, and *vice versa* (Frankel and Galun 1977). In the present study, it is presumed that morphactin might affect the balance of endogenous levels of these hormones either by enhancing the level of auxin/ethylene, or by decreasing the level of gibberellins. As a result, female flowers are produced on male plants.

Acknowledgement

The authors are grateful to the University Grants Commission, New Delhi, for financial assistance.

References

Atsmon D Lang A and Light E N 1968 Content and recovery of gibberellins in monoecious and gynoecious cucumber plants ; *Plant Physiol.* 43 806–810

Bisaria A K 1977 Effect of a morphactin on growth, sex expression, fruit set and yield in *Luffa acutangula* ; *Can. J. Bot.* 55 952–956

Bopp M 1972 On effect of morphactin. In *Hormonal regulation in plant growth and development* (eds.) H Kaldewy and Y Vardar (Weinheim : Verlag-Chemie) pp. 333–348

Bose T K and Nitsch J P 1970 Chemical alteration of sex expression in *Luffa acutangula* ; *Physiol. Plant.* **23** 1206–1211

Brewbaker J L and Kwack B H 1963 The essential role of calcium ion in pollen germination and pollen tube growth ; *Am. J. Bot.* **50** 859–865

Frankel R and Galun E 1977 *Pollination mechanism, reproduction and plant breeding* (Berlin, Heidelberg, New York: Springer Verlag)

Heslop-Harrison J 1957 The experimental modification of sex expression in flowering plants ; *Biol. Rev.* **32** 28–80

Jaiswal V S and Kumar A 1980a Sex reversal and fruit formation on male plants of *Morus nigra* L. by 2-chloroethylphosphonic acid ; *J. Exp. Bot.* **31** 497–500

Jaiswal V S and Kumar A 1980b Induction of male inflorescences on the female plants of *Morus nigra* L. by GA_3 ; *Indian J. Exp. Biol.* **18** 911–913

Krishnamoorthy H N 1971 Effects of morphactin on growth, and sex expression of *Luffa acutangula* ; *Z. Pflanzenphysiol.* **65** 88–91

Mohan Ram H Y and Jaiswal V S 1970 Induction of female flowers on male plants of *Cannabis sativa* L. by 2-chlorethanephosphonic acid ; *Experientia* **26** 214–216

Mohan Ram H Y and Jaiswal V S 1971 Feminization of male flower of *Cannabis sativa* L. by a morphactin ; *Naturwissenschaften* **58** 149–150

Mohan Ram H Y and Jaiswal V S 1972 Induction of male flowers on female plants of *Cannabis sativa* by gibberellins and its inhibition by Abscisic acid ; *Planta* **105** 263–266

Mohan Ram H Y and Jaiswal V S 1974 The possible role of ethylene and gibberellins in flower sex differentiation of *Cannabis sativa*. In *Plant growth substances* (ed.) Y Sumiki (Tokyo : Hirokawa Pub.) pp. 987–996

Robinson R W, Cantlifee D J and Shannon S 1971 Morphactin induced parthenocarpy in the cucumber ; *Science* **171** 1251–1252

Schneider G 1970 Morphactins. Physiology and performance ; *Annu. Rev. Plant Physiol.* **21** 499–536